

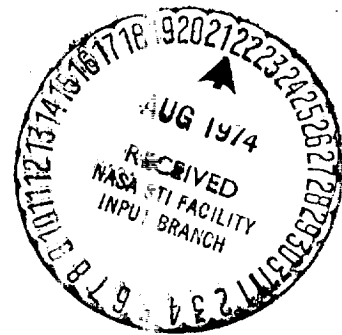
PRELIMINARY

ROUGH DRAFT

FUNGI ISOLATED FROM APOLLO ASTRONAUTS

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(NASA-TM-X-70151) FUNGI ISOLATED FROM
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Extensive mycological analyses of samples recovered from Apollo 14 and 15 astronauts have been conducted to satisfy two quite different objectives. One of these objectives was to establish, in as much as shifting microbial populations allow, a preflight mycological profile for each area and each crewmember sampled. This information was to be used as an aid in demonstrating the terrestrial origin of any micro-organisms isolated from returned lunar material. As viable micro-organisms were never recovered from lunar samples this objective was never exercised. The second objective was to obtain precise mycological data, under actual space flight conditions, which would identify significant shifts in microbial populations. Such changes in microbial flora have been shown, by T. D. Luckey and others, to present a potential hazard in space flight. In 1970 the Space Sciences Board of the National Academy of Sciences extensively reviewed this subject and showed that the results of numerous ground-based chamber and spacecraft simulator studies verify the potential hazard of fungi in space flights. The current study presents data which indicate the nature of observed mycological changes associated with the Apollo 14 and 15 crewmembers.

Methods and Materials

Twelve samples were collected from each astronaut as shown in figure 1 during each of the collection periods. Calcium alginate swabs, wetted in phosphate buffer, were used to sample the nostrils and each body surface area as shown in table I. Dry calcium alginate swabs were used to sample the throat prior to the collection of a gargle and mouth wash. A stool and a urine sample were also collected from each crewmember.

Swabs were placed in trypticase soy broth and maintained at 4°C for at least ten hours before processing. This delay was imposed in an attempt to equalize variations caused by shipment from remote collection sites. Following dilution of samples in TSB, aliquots of each sample were removed and streaked on specific mycology identification agar media, then incubated at 25°C for 120 hours. Other aliquots of the TSB sample were centrifuged at 12,000 X g (5,000 rpm) for 15 minutes. The resulting sediment was used to streak agar plates containing isolation media and antibiotics. All resulting colonies which were morphologically different from those isolated from the original plates were picked from the agar surface for identification. All isolates were identified to genus, and to species where possible, employing current keys.

Three samples were collected before launch as shown in table II. The same schedule was employed for both missions except that the intermediate samples (F-14 and F-5) were collected at different times. The immediate preflight samples (F-0) were collected the morning of launch, immediately upon arising. Immediate postflight samples were collected aboard the recovery ship and transported to MSC for analysis. The 16 day post-flight sample (R+16) collected for Apollo 14 was not duplicated on Apollo 15.

Results and Discussion

The fungi isolated from the Apollo 14 crewmembers were more varied than those recovered from the Apollo 15 astronauts. A total of 49 different

genera and 80 different species were recovered from the former throughout the mission, whereas only 32 genera and 52 species were recovered from the latter. If the extra Apollo 14 postflight sample set is subtracted, the data still show that 1/3 fewer different types of fungi were recovered from the Apollo 15 astronauts.

The mycological recovery from each sample period is presented in Figure 2. The data illustrated in this figure show that at the beginning of the study (F-30), a more diverse microbial load was recovered from the Apollo 14 crewmembers in that a total of 42 different species were present in the population (as compared to 34 for the Apollo 15 crew). The average number of different species per individual, however, was less with the Apollo 14 crew. These data indicate that whereas there was a greater variety of fungi on each of the individual Apollo 15 crewmembers, the microflora of the crewmembers were more similar to each other than were the flora of the Apollo 14 crew (or conversely, based on actual species, the Apollo 14 astronauts were more heterogeneous than were the Apollo 15 astronauts).

This initial sample was collected at the Manned Spacecraft Center 30 days before launch. Nine days later a regimen was initiated which restricted the prime and backup astronauts to several special areas at the Kennedy Space Center (KSC) which had been modified so that strict control of airborne microbial contaminants could be monitored. In addition, contact with contaminating fomites and food were guarded

against, and only a few persons were allowed access to the crewmembers. Another set of samples was collected during this health stabilization period as shown in Figure 3. These data show that the mycological load is significantly reduced during this period such that a preflight low of 21 different species per crew is reached by the morning of launch.

The Apollo astronauts are required to conduct training mission at several remote sites, including tropical areas, volcanos and confined test chambers. For this reason the authors feel that the 30-day preflight values are unusually expanded and are not representative of less-traveled persons. The preflight decrease in mycological load probably reflects a stabilization of the normal flora with a loss of transients and a reduction of common occupants. The immediate preflight value is probably unusually depressed as a result of the incarceration in a super clean environment for 21 days with only minimal contact with possible contaminating objects.

A similar analysis, conducted by Levine and others with Tektite I subjects indicates that the fungal load remained quite stable for 38 days prior to a dive when no attempt was made to isolate the crewmembers. In studies where closed environments have been employed, however, similar stabilization of the microbial population is often observed.

Analysis of immediate postflight Apollo samples show that the observable flora were reduced to 1/3 or less of the immediate preflight value.

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This probably reflects an equilibrium shift mediated by the spaceflight environment. During the flight the crew were in an environment of nearly pure oxygen at a pressure of 1/3 atmosphere. At the beginning of the flight the internal hardware components were quite clean and the astronauts exercised a rigorous hygiene regimen throughout the mission. These factors could be expected to upset the established equilibrium whereby the astronauts lost cells to, and picked up cells from, the environment. The data show that the equilibrium was definitely shifted to favor loss of fungi from the body.

A set of samples was collected 16 days following recovery of the Apollo 14 astronauts. Analysis of these specimens demonstrated that the number of different fungal species returned to just slightly higher than the preflight low. During this 16-day postflight period the crew were maintained in total isolation within the Lunar Receiving Laboratory but were in close contact with 18 attendant personnel. These data suggest that the equilibrium had shifted back to a more normal mode and that the crew were not yet subjected to the high influx of fungal species mediated by their premission travels.

The above observations are based on data gathered from 7 body surface swabs, a throat swab, a gargle sample, a stool, and a urine sample. An analysis of which of these areas were most affected by the mission environment is presented in Figure 4. These data show that the loss is most prominent from the skin areas, which is to be expected. In fact

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this loss was so great that 86% of the Apollo 14 immediate postflight isolates were recovered from the gargle and stool. Further, species variation was decreased so much that 90% of the immediate postflight isolates from the two combined missions belonged to the three genera Rothia, Candida, and Penicillium.

Summary

TABLE I CREW SAMPLE COLLECTION

<u>Sample Designation</u>	<u>Area Sampled</u>
Scalp	2 sq. in. above hairline at base of neck
Ears	Right and left external auditory canals with two revolutions of each swab in each canal
Axillae	1 sq. in. below hair area on each side
Hands	1 sq. in. on right and left palms
Navel	The internal area of the umbilicus, and surrounding 2 sq. in. area with at least two revolutions made with each swab.
Groin	2 in. strip from rear to front on right and left inguinal area between legs
Toes	Area between large and first toe of each foot
Nares (Nasal Swab)	Both nostrils
Throat Swab	Surfaces of tonsils and posterior pharyngeal vault swabbed with each of two dry calcium alginate swabs
Gargle	60 ml phosphate buffer used as gargle and washed through oral cavity three times
Urine	60 ml midstream sample
Feces	Two samples of 100 mg each taken from center of the fecal specimen

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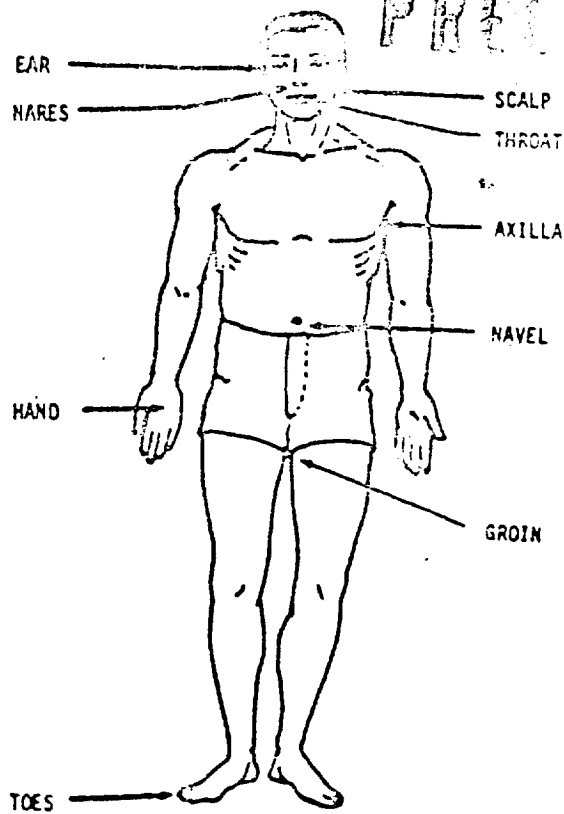
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Table II Crew Microbiology Sample Schedule

Apollo 14	F-30	F-14	---	F-0	R+	R+16
Apollo 15	F-30	---	F-5	F-0	R+	----

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Ganglia
Stool
Urine

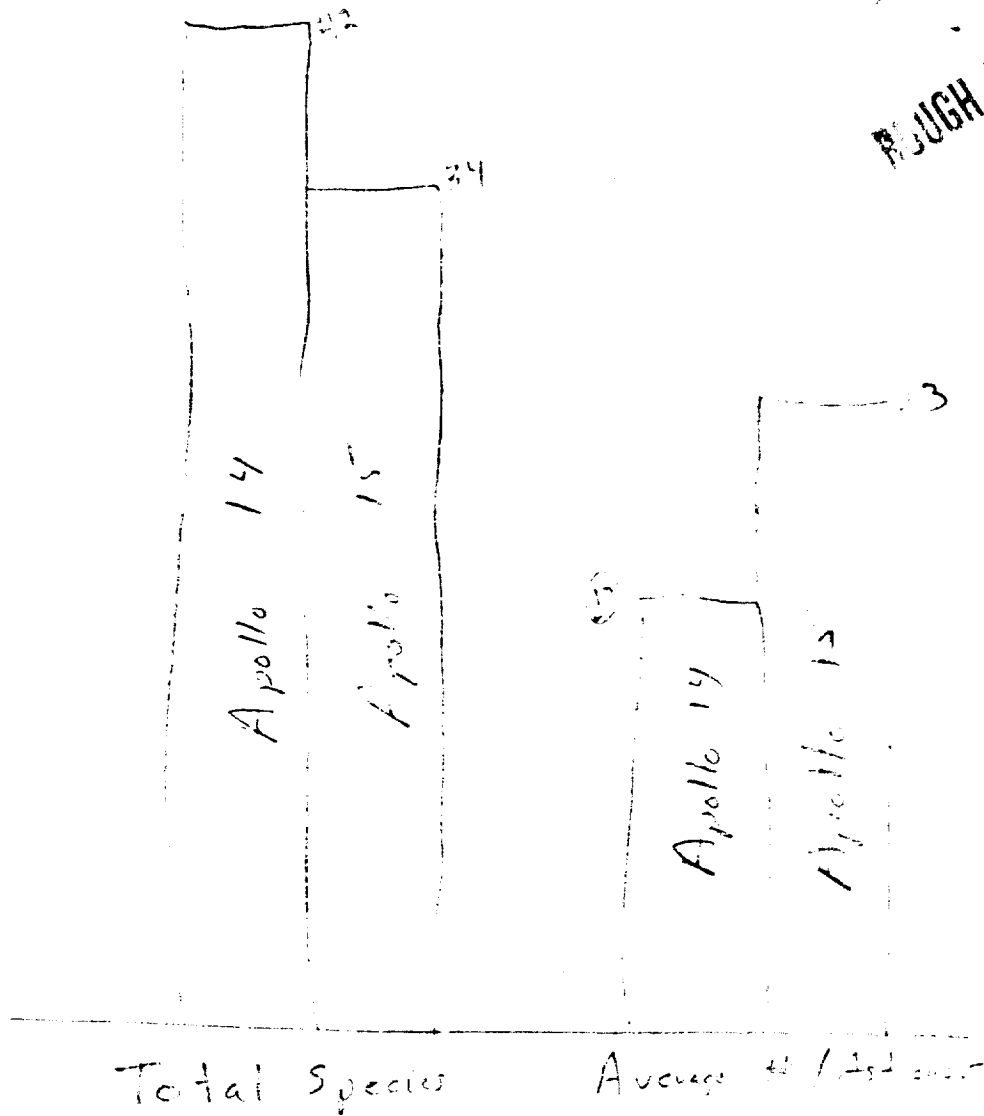
Figure 1. - Swab Sampling Areas.

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Figure 2 Mycology Isolates Recovered
From Apollo Crewmembers (F-30 Env.)

of Species Recovered

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Project Solution

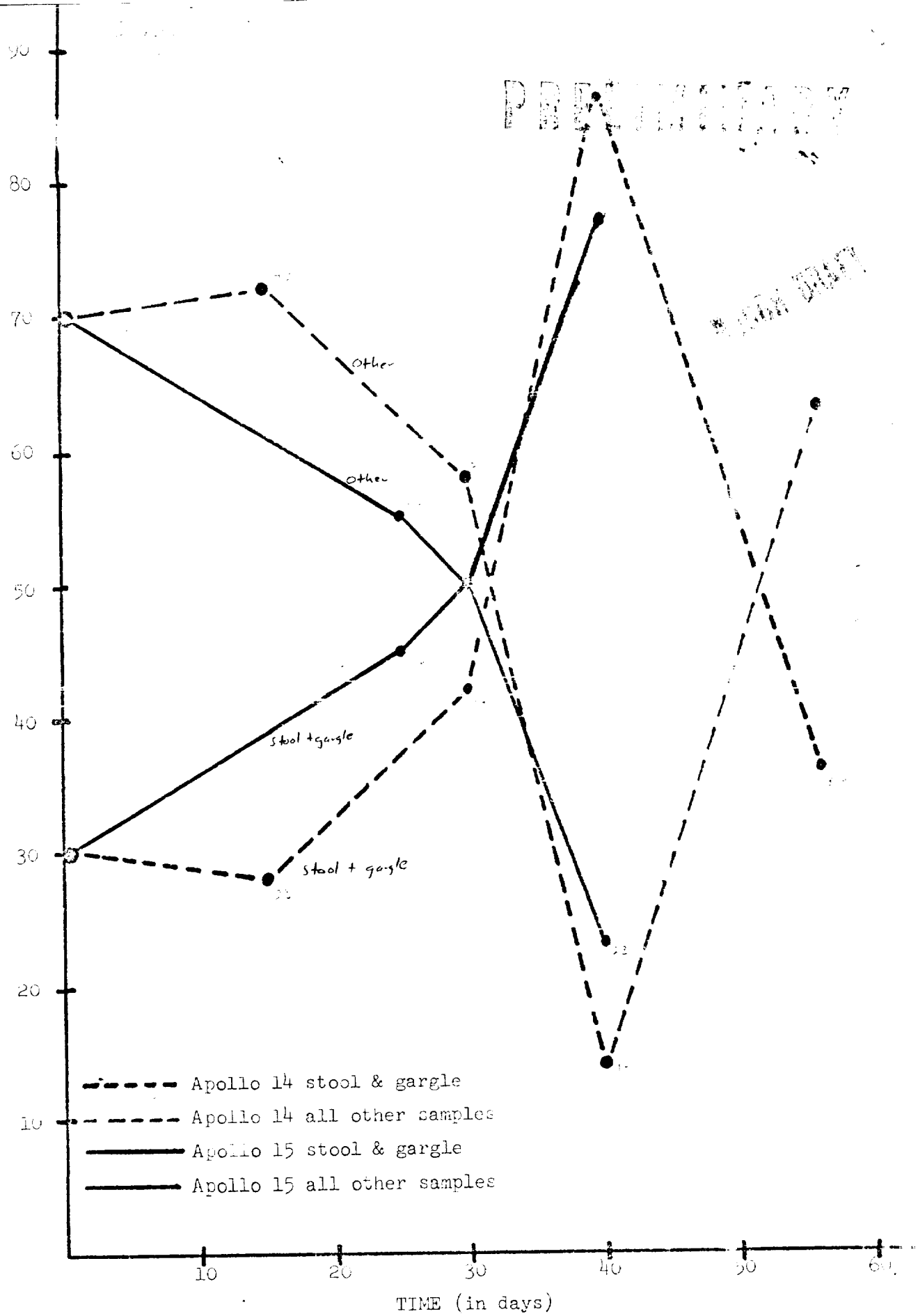
Flight

Project Solution

11/10/14

11/10/14

DIFFERENT SPECIES ISOLATED (in percent of total)



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FOR DRAFT